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REVIEW

Tumors in Amphibia

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INTRODUCTION

Amphibians, together with reptiles and fishes, are poikilothermic (or ectothermic) animals and are often referred to as lower vertebrates. The principal body structures and organs of lower vertebrates, however, are the same as those of the higher vertebrates such as birds and mammals. It is not surprising, therefore, that tumors of amphibians with regard to the organs affected by the tumor, the morphology of the tumors and the relationships between factors of tumorigenesis and tumor formation, are basically the same as those of other vertebrates, including the human being. Many investigators have been studying tumors mainly in mammals and this, no doubt, has increased our knowledge concerning tumors; but at the same time aspects of comparative oncology within different vertebrate classes have been somewhat neglected despite a certain amount of solid fundamental works [1–4].

Recognising the unique position of amphibians as animals of both aquatic and terrestrial habitats we and others before us [5–16] have been looking for tumors, their causes, their growth patterns, etc. in these animals for quite some time now. Although amphibians have long been used as suitable material for various lines of research within the subject of biology, e.g. experimental embryology, biochemistry, genetics and cell biology, the number of reports on tumors in amphibia is relatively small. In mammals, birds and fishes, on the other hand, many different kinds of tumors have been studied and the number of reports is larger.

There are two major explanations for the small number of reports on amphibian tumor. One is that investigators, who have used amphibians as experimental material for all kinds of research, may not specifically have looked for tumors and, thus, may not have reported them [17]. Tumors could have been overlooked and if investigators, dealing with amphibians, had carefully examined the internal organs of amphibia, various types of tumors could possibly have been found. The other explanation is that amphibians do suffer less from tumor, because they may have some specific tumor-repelling system which makes it especially hard for a tumor to form in their bodies. In short, they may be different from all other vertebrates with regard to tumors [18, 19]. If such a characteristic or something in the nature of amphibians is present that prevents tumors from forming or proliferating we have to identify these forces and relate them to the characteristic way of life of amphibians. For example, amphibians can live both in water and on land; they highly depend on the changes of the environment; as adults they are entirely carnivorous, etc. In addition to the ability to adapt to the environment, they have other characteristics such as metamorphosis during development and, especially in urodela, a remarkably strong ability to regenerate lost or damaged parts of the body. When we consider

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tumors of amphibians in relation to their unique biological characteristics, on the one hand, and their similarity to human neoplasms on the other [5]. Amphibians may perhaps turn out to be particularly suitable material for tumor investigation. Already Khudoley has been calling *Rana temporaria* "a new experimental animal in cancer research" [20]. The one amphibian tumor that has been well investigated and has been known for decades is renal adenocarcinoma in *Rana pipiens* first reported by Lucké in 1934 [21]. In addition to furthering our knowledge of tumor generally, the purpose of our study on tumor of amphibians is to recognise common problems in cancer research and to study the biological nature of tumor by making use of the unique biology of one group of vertebrates: amphibians.

Recently, papillomata in the newt *Cynops pyrrhogaster* and tumors in *Xenopus laevis* have been found and investigated. In this review we intend to summarize what is known about tumors in amphibia, concentrating on studies of renal adenocarcinoma in *Rana,* papilloma in *Cynops* and tumors in *Xenopus.*

REPORTS ON SPONTANEOUS TUMORS

The number of reports on spontaneous tumors in amphibians is smaller than that dealing with tumors in mammals, birds and fishes. For exam-

Table 1. List of spontaneous tumors in anurans (—1986)

Species	Tumors (number of animals)	Sites
Rana pipiens	osteogenic sarcoma (1), adenocarcinoma (many) carcinoma (6), teratoma (1), lymphosarcoma (3), liposarcoma (2), epithelioma (1), hepatoma (2), mesothelioma (2), carcinosarcoma (1), rhabdomyosarcoma (1), plasmacytoma (1), cystoadenocarcinoma (1), squamous cell carcinoma (7), papilloma (1)	thigh, kidney, lung, fat body, muscle, ovary, spleen, bladder, viscera, skin, liver, dermal glands,
Rana esculenta	carcinoma (1), fibroma (1), adenoma (1), hepatoma (1), adenocarcinoma (1), hypernephroma (1), sarcoma (1)	buccal cavity, kidney, ovary, leg, liver
Rana catesbeiana	adenocarcinoma (2), neurosarcoma (1) adenoepithelioma (2)	skin, sacral plexus, kidney
Rana clamitance	myxosarcoma (1)	tail
Rana temporaria	melanoma (1), epithelioma (1) cystadenocarcinoma and cystadenopapilloma (7)	skin
Rana arvalis	adenoma (1)	skin
Rana ridibunda	cystadenocarcinoma and cystadenopapilloma (16)	skin
Rana chensinensis	tumor-like displasias (many)	limb
Bufo bufo	capsulated tumor (1), fibroma (8), lipoma (1)	kidney, skin, bladder
Bufo calamita	adenocarcinoma (1)	lung
Bufo marinus	adenoma (1)	parotid gland
Bufo boreas	fibroma (1)	muscle
Ceratophrys ornata	fibrosarcoma (1)	leg, kidney
Dendrobates pumilio	erythrophoroma (1)	viscera, skin
Hyla meridionalis	guanophoroma (1)	skin
Hyla arborea	xanthophoroma (1)	skin
Xenopus laevis	lymphosarcoma (3), carcinoma (1), fibroma (1), adenocarcinoma (3), fibromata (1), nephroblastoma (1), lipoma (1), papilloma (1), adenoma (1), melanoma (8), neuroma (3)	kidney, pelvis, face, under skin, viscera, head, skin, liver, orbit
Xenopus fraseri	lymphosarcoma (2)	viscera

Table 2. List of spontaneous tumors in urodeles (—1986)

Species	Tumors (number of animals)	Sites
Andrias japonica	fibroma (2), carcinoma (1), fibroma (1)	limb, testis, under skin
Ambystoma opacum	mixed tumor (1)	skin
Ambystoma tigrinum	papilloma (1), fibroma (2), melanoma (1), melanocytoma (1), myxofibroma (1)	skin
Ambystoma mexicanum	melanoma (4), lymphosarcoma (3), melanosarcoma (1), epithelioma (1), adenocarcinoma (1), neuroepithelioma (2) teratoma (1), testicular tumor (16)	skin, mouth, tail, testis
Amphiuma tridactylum	leiomyoma (1)	lung
Necturus maculosus	adenocarcinoma (1)	kidney
Triturus cristatus	adenocarcinoma (1), melanoma (1)	skin gland, skin
Triturus alpestris	carcinoma (4), epithelioma (1)	skin
Triturus vulgaris	chondroma (1), fibroma (25)	skin
Cynops (=*Triturus*) *pyrrhogaster*	lymphosarcoma (1), sarcoma (5), papilloma (many), nephroblastoma (1)	viscera, liver, skin, kidney
Notophthalmus viridescens	mesenchymal tumor (1), neuroblastoma (1)	skin, under skin, neck
Cryptobranchus alleganiensis	adenoma (1)	testis

ple, Effron *et al.* examined tumors by necropsy and by histology in various species of wild animals which had died in the San Diego Zoological Garden and in the Wild Animal Park region from 1964 to 1976 [22]. They found tumors in 2.75% of 3,127 mammals, 1.89% of 5,957 birds and 1.90% of 1,233 reptiles, but they did not detect any tumors in amphibia (0% of 198).

The number of reports on spontaneous tumors in amphibians known to us up until 1986 is about 491 cases involving 18 species of anurans and about 253 cases involving 12 species of urodeles. Relevant data are listed in Tables 1 and 2. The known amphibian tumors are dealt with in some excellent reviews [8, 11, 17, 22–28]. The number of reports in anurans is greater than that in urodeles which according to Brunst [29] is merely a reflection of the greater extent to which anurans are used in research. In some anuran species like *Rana pipiens, Rana esculenta* and *Xenopus laevis* several kinds of tumor were reported; the same holds true for the urodele species *Ambystoma tigrinum, Ambystoma mexicanum* and *Cynops pyrrhogaster.* These species are frequently used as experimental material in biology. Amongst the tumors listed in Tables 1 and 2, there are some reports dealing with precancerous changes and tumors in *Rana.* Tumors in intersubspecific and interspecific hybrids have also been reported, e.g. in *Xenopus laevis laevis* × *Xenopus laevis victorianus* and *Rana pipiens* × *Rana palustris.* Lymphosarcoma has been found in the viscera of the former frog, which was produced by nuclear transplantation. The tumor in the latter was teratocarcinoma in the testes. The reported tumors were classified into six types; epithelioma, mesenchymal tumor, pigment tumor, blood cell tumor, central nervous system tumor and reproductive organ tumor. Epithelial tumors such as adenocarcinoma, adenoma, and papilloma accounted for nearly half of all reports. Epithelial tumors are thought to be related to the fact that amphibians have many glands in the skin and that the skin is covered by a thin *stratum corneum.* Mesenchymal tumors, such as fibroma, lipoma and smooth muscle tumor, made up about a quarter of the total. Tumors of pigment cells, hematopoietic cells, and gonads could also be encountered. Tumors of the central nervous system were reported once each in anurans and urodeles. It is very rare that the same type of tumor has been found in many animals of the

same species, but renal adenocarcinoma in the leopard frog *Rana pipiens* (Lucké renal tumor) and papilloma in the Japanese newt *Cynops pyrrhogaster* (newt papilloma) are exceptions and have been found in very large numbers. These tumors will be discussed later.

Recently, a few reports on the incidence of spontaneous tumors in amphibians have been published. Khudoley and Mizgireuv collected 320 specimens of *Rana temporaria* and 978 *Rana ridibunda* in the Leningrad region and found tumors in 7 and 16 frogs, respectively [30]. There were one to seven tumors in each frog and the tumors were all cystadenopapillomata or cystadenocarcinomas originating from mucous glands. Infiltrations were not found in most animals, but a metastasis was found in one frog. Mizgireuv *et al.* collected many frogs and toads in three regions of Southern Sakhalin [31]. They found tumor-like dysplasiae of osteochondrous tissue of hind limbs in *Rana chensinensis*. With 11.5% out of 1,095 frogs the highest incidences of the dysplasiae were observed in point A, a place polluted with the sewage effluent of a paper factory; in points B and C the figures were 5.5% of 3,651 and 0% of 1,614, respectively. Oinuma *et al.* observed large tumors in the dorsal region of four adult females of the African clawed frog (*Xenopus laevis*) [32]. The tumor-bearing frogs were found in amongst about 20,000 adults which were bred in artificial ponds. Surprisingly, no tumors were seen in about 10,000 larvae and 5,000 juvenile frogs. The tumors of the four frogs were similar to each other and were thought to be melanomas and neuromas. In urodeles, Counts *et al.* reported a mixed tumor in a male *Ambystoma opacum* [33]. The number of collected animals was not clear. The tumor was composed of epithelial cells and mesenchymal cells and it appeared rather benign. Khudoley and Eliseiv found a melanoma in the skin of one out of 272 axolotls (*Ambystoma mexicanum*) of 5 months old [34]. This tumor proliferated and metastasized during breeding. Counts *et al.* captured about 300 newts (*Notophthalmus viridescens*) and found a neuroblastoma in one newt [35]. A cyst of connective tissue was observed and the tumor occurred under the skin.

Deformations and abnormal growth in amphibians have repeatedly been reported [36, 37]. In 1969 Rostand and Darré suggested that deformations such as brachymelia, polymelia, and polydactylism in *Rana esculenta* could have been caused by a teratogenic virus, which was carried by certain species of fish like tench and eel [38]. These two authors, thus, appear to have been one of the first to recognise the connection between virus and abnormal regeneration. Other possible causes of abnormal growth must, of course, not be completely discounted [37].

FREQUENCY OF TUMOR OCCURRENCE

We have seen in the preceding section that some kinds of tumor in anurans and urodeles are nothing new and have been known for quite a while. However, the types of tumor which could be used or have been used as detailed experimental material for detailed investigations are very few in number. The three kinds among them which we shall discuss one by one are Lucké renal adenocarcinoma in anurans, melanoma and neuroma in the African clawed frog *Xenopus*, and newt papilloma in urodeles. An important point to consider is also the artificial tumorigenesis in amphibians using carcinogenic materials.

1. Lucké renal tumor

Lucké renal tumor is an adenocarcinoma in the kidney of the leopard frog (*Rana pipiens*) and was first reported by Lucké in 1934 [21]. Since then, this tumor has been investigated as a model of tumors in lower vertebrates. Lucké renal tumor appears spontaneously at a relatively low frequency (less than 13%) in one kidney alone or on both sides. Almost no metastases to other organs were observed. However, when the frogs were kept in a laboratory (at about 22°C) for a long period the incidence of spontaneously appearing tumors increased by about 50%, and metastases to lung or liver became recognisable. The origin of the tumor cells is considered to be the epithelium of the urinary tubule, since microvilli were often observed in the tumor cells. The transplantation to healthy frogs is possible. When the tumor fragment was implanted into the anterior chamber of the eye, the transplants proliferated very rapid-

ly [39, 40] and might induce formation of a tumor in the kidney of the host.

McKinnell *et al.* examined seasonal fluctuations of the Lucké renal tumor from 1965 to 1968 [41, 42]. They collected a total of 3,367 frogs from the wild in spring, summer and autumn, and found that the tumor-bearing frogs were most numerous in spring and autumn (average 5.0% and 4.4%, respectively), but considerably less so in summer (0.14%). As for the reason of the rare appearance of tumorous frogs in summer, McKinnell *et al.* considered that the tumor-bearing frogs were easily captured by their predators and, thus, showed a lower survival rate, but they could not exclude the possibility that death of sick animals or spontaneous regression of the tumor were additional, important factors involved. McKinnell *et al.* [42] collected a total of 1,363 frogs at 15 localities in Minnesota and it became obvious that the frequency of the tumorous frogs depended not only on seasons as described above, but also on the specific region in Minnesota from which the frogs came. In spring or autumn collections, tumor-bearing frogs made up 0.9–14.0% in 9 out of 15 regions, but no tumor-bearing frog was found in any of the other 6 regions.

Thereafter, McKinnell *et al.* turned towards the phenomenon of decreasing numbers of tumor-bearing frogs in Minnesota [43, 44]. In the regions where many tumor-bearing frogs had appeared in the springs and autumns of the years 1966–1975, they captured 685 frogs in 1977 and 1,216 in 1978 and 1979, but not one tumor-bearing frog was found. Nowadays it appears to be difficult to find renal adenocarcinoma in *R. pipiens* from the wild, but a full explanation why this should be so remains to be put forward.

2. *Tumor in Xenopus laevis*

Throughout the world the African clawed frog (*Xenopus laevis*) has been used as an experimental animal for a wide range of biological investigations. However, as stated in Table 1, reports on tumors number only 24 cases. This figure seems to indicate a very low incidence. Oinuma *et al.* examined the frequency of tumor in *Xenopus* bred in an artificial pond in 1983 and 1984 [32]. In the first examination, they found four tumor-bearing frogs in a population of about 20,000 frogs (0.02%). But following re-examination 7 months later, no tumor-bearing frog could be found at all. Compared with the cases of Lucké renal tumor or newt papilloma the frequency of tumor in *Xenopus* is really considerably depressed. Considering Lucké renal tumor and newt papilloma, normal frogs or newts kept with tumor-bearing animals of the same species in the same water tank for a long period (about a year) developed the tumor with high regularity (more than 50%). However, as for the tumor of the African clawed frog, it did not occur in normal animals for at least a year. It seems that the infection is related to a virus and that the path of the infection takes in *Xenopus* is different from that of Lucké tumor or newt papilloma. In tissue sections of the tumor in *Xenopus,* many mature pigment cells were observed among the tumor cells. The DOPA test was carried out on the tumorous tissue, and positive results were obtained [28]. Based on these results and the long term-cultivation of these cells [45], the tumors in *Xenopus* were thought to be melanomas and neuromas.

3. *Newt papilloma*

Papillomata are occasionally found in the skin of the Japanese newt, *Cynops pyrrhogaster.* The tumors may be found anywhere on the body surface but preferably occur on the tail, back, and limbs. This tumor, which is also called epithelioma, was first reported from one case each by Honma and Murakawa [46] and Bryant [47]. Since then newt papilloma has been found in large numbers in a variety of species (e.g. *Diemictylus viridescens*: [48]; *Triturus alpestris*: [49]; *T. cristatus*: [10, 50]) and presently newt papilloma as well as Lucké renal tumor is considered to represent important material for the investigation of amphibian tumor [51–56]. As newt papilloma often regresses and disappears during breeding, it may turn out to be very suitable material to analyse the mechanisms involved in the spontaneous regression of the tumor. Newt papilloma grows by proliferation of epithelial cells of the tumor region, but metastases have not been observed in any organs other than the skin.

Asashima *et al.* collected newts at specific localities in Niigata prefecture from autumn of 1979 to autumn of 1983 and have examined the seasonal changes of the papilloma [53, 54].

The total number of newts collected was 28,630 and the frequency of papilloma-bearing newts among the collected newts was monitored for every season. Papilloma-bearing newts were numerous only in autumn (1.9–7.9%) whereas in spring, summer and winter (0.16–0.32%, 0.47–0.50% and 0.48–0.50%, respectively) they were far less frequent. Though males tended to be affected more commonly than females, it is not clear if there really is a sex-related difference in the abundance of papilloma. Seasonal peaks in papilloma-bearing newts not only occurred in Niigata but in definite regions of other prefectures (Yamagata and Iwate), too.

The incidence of Lucké renal tumor was high in two seasons per annum, namely, spring and autumn, but that of newt papilloma was high only in autumn. The reason for this difference is not easy to understand.

Besides seasonal changes in the abundance of papilloma-bearing newts, geographical variations of the tumor frequency in autumn from 1980 to 1985 have also been examined [53, 54]. Newts from 16 prefectures in Japan were collected and examined whether they had papillomata or not. Papilloma-bearing newts were numerous in Aomori, Iwate, Yamagata and Niigata prefectures (1.3–7.9%), less numerous in Yamanashi, Gifu, and Shimane (0.7–2.6%), and least numerous in Chiba, Shizuoka, Aichi, Kochi, Okayama, Nagasaki, Saga, Kumamoto and Miyazaki (0–0.6%). More newts from the North and West side of Japan than from Southern and Eastern parts suffered from papillomas. To complicate the picture, there are prefectures like Kochi and Shizuoka in which papilloma-bearing newts were found in some years but apparently seemed absent in others.

THE RELATION BETWEEN TUMOR AND ONCOGENETIC FACTOR

1. Oncogenic virus

The existence of viruses in amphibian tumors has been proved in some cases. It became evident, for example, that Lucké renal tumor was caused by a virus [3, 8]. In newt papilloma, too, virus particles were detected [51, 53] and spontaneous tumors have successfully been transmitted by experiment (e.g. in *Pleurodeles waltii*: [57]). In *Xenopus* lymphosarcoma virus particles were detected and infection experiments gave equally positive results. Particles resembling viruses were also present in *Xenopus* melanoma. Fish-born viruses were thought to be involved in abnormal growth of appendages in *Rana esculenta* [38].

For Lucké renal tumor there no longer exists any doubt that it is caused by a virus [35, 58, 59]. Lucké on the basis of the following observations had already suggested that the tumor was caused by a virus; the acidophilic inclusion bodies existed in the nucleus of the tumor cells and resembled those agents responsible for *herpes* infection [21]. When glycerinated or dried tumor was inoculated to another, healthy leopard frog, the tumor appeared regularly soon after [58]. At present, this virus is known as Lucké herpes virus. Though the virus was present in most instances, there were cases in which it was not found. Rafferty noticed that the virus was found in tumors of frogs captured in winter, but not in summer material [40]. Subsequently the virus was observed by electron microscopy in the renal tumor of a frog captured in winter, and in the tumor of a frog kept at low temperature in the laboratory.

Actually, when seven tumor-bearing frogs in hibernation were exposed to a higher temperature (20–22°C), many inclusion-body containing cells were observed at first but later they were broken and the residues of cells and virus particles were flushed out into the urinary tubules. Seven days later, cells that did not contain any inclusion-bodies were to be observed in the tumor [60]. On the other hand, when frogs were captured before they entered a lake for hibernation and they were put in a cage and experimentally immersed in the

lake [61], the virus was not found in the tumor before the frogs entered the lake, but began to appear seven days later so that more than a month later all the tumors contained viruses. The water temperature in the lake was 5–9°C. Even though tumor-bearing frogs were kept at low temperature in the laboratory, a similar result could not be obtained. Thus, the maturation of the virus appears to occur at low temperature while the proliferation of the tumor cells requires higher temperature. The encapsulation of Lucké herpes virus is a hibernation-related phenomenon in nature. Though the virus is not found in tumors of the summer-type, it is believed that the virus genome is contained in the tumor cells in a masked or latent state [40].

When frog larvae were reared segregated from early stages of development, the frequency of tumor formation was almost the same as that found in the field. The infection by virus from a tumor-bearing frog to another healthy animal may occur at an early period of development [40]. Furthermore, such an infection seems to occur perhaps in spring, the season of spawning and embryonic development. Horizontal infections might well represent one pathway for the spread of the disease, because experiments with transmitting infections have been successful.

Generally, to know whether a virus is a tumor agent, transplantation experiments have to be carried out successfully. Cultured cells will have to be infected with the virus and their transplantability has to be affirmed. In Lucké tumor, it has been proved that the virus was the etiological agent on the basis of virus isolation, transplantation and cell culture studies [96].

Tweedell separated the tissue of frog renal tumor into cell organelle fractions which were kept under low temperature [62]. Each fraction was injected to sterilized early embryos or hatching larvae. In embryos injected with the mitochondrial fraction, tumors were formed in the pronephros or mesonephros in large numbers (13–92%) during or after metamorphosis. In the embryos injected with the microsomal fraction, tumors were also induced in the metamorphosing larvae or in juvenile frogs (0–50%). These newly created tumors were proliferating renal adenocarcinomas. Furthermore, Mizell separated the mitochondrial fraction of the tumor into several fractions by the zonal centrifugation method and obtained a fraction which readily induced the growth of tumors when it was injected into early embryos [63]. The establishment of cell lines originating from the virus-induced pronephric tumor was performed by Tweedell and Williams [64]. Primary explants obtained from normal pronephri of larvae were cultured *in vitro* and the cells were infected with herpes virus obtained from adult tumors. These cells were cultured for three passages and the mitochondria-herpes virus fraction was obtained from these cells. Then frog embryos were inoculated with the fraction. When the embryos developed and became tadpoles, the tumor was formed in pronephros or mesonephros. Dissociated cells were obtained from this tumor, cultured, and two cell lines PNRT 4 and PNRT were established. More than 85% of these cells were epitheloid and the rest was fibroblastic.

Naegele *et al.* examined whether Lucké herpes virus fulfilled Koch-Henle postulates [65]. According to Koch-Henle postulates, the experiment was separated into 4 steps. (1) Herpes virus was associated with kidney tumor of frog adult. (2) A cell fraction, containing virus, was obtained from the tumor. Tail-bud embryos were infected with this fraction and allowed to develop until tadpoles. Tumors were induced in pronephros or mesonephros of these tadpoles at a high frequency (about 62%). (3) Then the tissue fragments of the induced tumor were cultured. If the tissue was kept at 7.5°C, herpes virus was detected in the nucleus, but if kept at 22°C, the virus was not found. (4) Cultured tissue fragments were homogenised and centrifuged. Early embryos were inoculated with the supernatant and were allowed to develop. Tumors were not induced in animals inoculated with the cell extract kept at 22°C, but readily so (64.7%) when they received the cell extract kept at 7.5°C. Herpes virus was detected in the newly formed tumor. It had oncogenic activity and it was the same virus isolated from the tumor of wild adult frogs.

Nace *et al.* found an antigen "X" by immunoelectrophoresis and fluorescent antibody techniques, which was contained in normal cells

but was absent from tumor cells. The antigen X was identified as a lysozyme [66]. There were at least eight isozymes in the normal kidney of adult frogs. One of them always existed, three were absent from the tumor and others were variably distributed. Since frog lysozyme was thought to possess activity against frog herpes virus [67], the hypothesis was advanced that the absence of an isozyme of lysozyme was linked to the virus infection and the subsequent initiation of the growth of the tumor.

Next, as for the newt papilloma, the existence of virus particles was confirmed in the tumor by Pfeiffer *et al.* [51] and Asashima *et al.* [53]. Which type of virus group this virus belonged to has not been clearly established yet. This virus resembles the herpes virus and Lucké herpes virus in regard to size and morphology but it is entirely possible that newt papilloma virus belongs to the group of iridoviridae [68]. Because the virus is often observed to proliferate in the cytoplasm of papilloma cells, the core of the virus particle must be large and the form of the virus a 6-edged body. This has to suffice to determine the nature of this virus and the nature and size of nucleic acid of this virus. Isolation of it will be required.

In newts papilloma transplantation experiments were successfully carried out [69]. When tumor fragments were implanted under the skin of healthy newts, tumors were formed in the skin of 17% of the hosts within a year. The tumorous tissue was then homogenized. When this homogenate was used for inoculation in newts collected at Kumamoto prefecture, a region where tumor-bearing newts had not been found previously, tumors did occur in 10.0–15.8% of these newts. In these transplantation studies, the frequency of tumor formation was higher than that of the control experiments and that of newts captured from nature. During the study of tumor homogenate injection, it became obvious that we did not know whether all the cells of the tumor were destroyed completely, but at least it appears to be the generally accepted view now that some factor(s) contained in the tumor cells induce the tumor.

Differences in the protein patterns of papilloma and normal skin tissues of the Japanese newt *Cynops pyrrhogaster* were studied by the two-dimensional (2–D) gel electrophoresis method. Also compared were protein patterns of skin derived from different regions of the same body and that from male and females. Groupings of 11 protein spots specific to normal skin and 7 protein spots specific to papilloma were detected [70]. The papilloma specific protein spots were not detected in the normal skin of adult newts, the skin of larvae, the presumptive ectodermal region of embryos, or such organs as the lung and liver. There were some differences regionally, but none by sex. One of the 11 spots specific to normal skin of adult newts was found to coincide with one in the spot grouping of larval skin. Two unique spots were identified in normal larval skin. The possibility exists that the appearance of papilloma specific proteins indicates the presence of virus associated proteins.

Virus particles were also seen in at least two cases of tumors of *Xenopus laevis*. In the first case, virus particles were detected in lymphosarcoma by electron microscopy [71]. A cell free extract containing the virus of this tumor could induce tumor formation. This infectious virus was about 0.05 μm in size. In the second case, a virus was found in a melanoma of *Xenopus laevis* [32]. The virus particles were often found in large number in the nucleus of a tumor cell, and in some cells, it was observed, that the inside of the nucleus was filled with virus, which would actually spill over into the cytoplasm. The virus found in the melanoma of *Xenopus* was considered to be herpes virus judging by its morphology, size and situation of the core.

The existence of virus associated with tumor in amphibians must, therefore, be regarded as factual. From now on amphibian virus-associated tumors could provide material for the analysis of tumorigenesis, cell transformation and cell differentiation and for the comparative study of the virology of tumor.

2. *The effects of carcinogens*

A large number of abiotic causes for tumorous growths have become recognised, notably ionising radiation and chemicals. The latter may exert their effectiveness directly or indirectly through food

uptake and the production of carcinogenic breakdown-products.

When a carcinogenic chemical, known to induce tumor in mammals and fish following exposure to a small quantity or dose of it, is given to amphibians, the probability of inducing tumor is generally low [27, 28, 72].

Methylcholanthrene (MC), which is known to induce skin tumor when applied to the skin of mice and which induces sarcoma when injected subcutaneously, exerts an effect that is broadly the same in both anurans and urodeles. 3, 4-Benzopyrene (BP) showed similar results. BP induces only skin tumors in urodeles but tumors of internal organs in anurans. Though hepatoma was induced with dimethylnitrosoamine (DMNA), diethylnitrosoamine (DENA), benzidine and aflatoxin in anurans, no hepatoma was formed with these carcinogens in urodeles.

It is thought that urodeles have strong internal resistance to the hepatoma-causing carcinogens. The authors examined newts (*Cynops pyrrhogaster*) which were bred in water containing a carcinogen such as 4-nitroquinoline-1-oxide (4-NQO), N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) or DMNA for a long period (a year), but no tumor formation was found in any organ of these newts (data not shown). It is suggested that urodeles possess some kind of system which minimises the effect of the carcinogens.

Regeneration phenomena are known to occur in urodeles following amputation of a limb or the tail. Though a carcinogen such as MNNG or 4-NQO was administered into the regeneration blastema, no tumor formation was observed and the differentiation itself proceeded normally, though various abnormalities (=morphological malformations) occurred. This problem will be discussed later.

It seems certain that amphibians, and in particular urodeles, are less sensitive towards carcinogens than other vertebrates. Since urodeles are the least sensitive, they could possibly serve in conjunction with their strong regeneration capacity as a convenient experimental animal in the study of the mechanisms of cellular resistance against carcinogens.

BIOLOGY OF TUMOR CELLS

The last published work of the Nobel laureate Hans Spemann, the discoverer of the organiser in the amphibian embryo, dealt with the tumor problem [73]. He transplanted the organiser region of gastrula stage embryos onto the liver of the adult newt *Triton taeniatus* and finally found the tumor cells resembling those of teratocarcinoma near the transplanted area of the newt. Following this experiment, many other investigators have used the amphibian tumor cells to study aspects of cell differentiation.

1. The relation between regeneration and tumor cells

In amphibians, the number of reports on spontaneous tumors is less than that for other vertebrates, and the resistance of amphibians towards chemicals, which in other animals are known to possess strong carcinogenic activity, seems to be very high. If it is true that in amphibians tumors have greater difficulty to form, then this may well be related to the strong regeneration potential of amphibians. Urodeles, in particular, have an enormous capacity for regeneration. When limbs, tail, or lens are amputated or removed, the powers of regeneration in each amputated area begin to work to reconstruct the original morphology. Dedifferentiation, proliferation, redifferentiation and morphogenesis occur in the amputated region, which eventually is restored to the former state. Tumor cells, however, escape from the contact of normal cells and from the control in normal tissues, and they proliferate independently and abnormally ignoring the order of the surrounding tissue. Though the cells in the "regeneration field" proliferate abnormally, their degree of freedom is minimised by the effect of the "regeneration field" in the rest of the amputated region, which is also involved in the process of normal cell differentiation.

It is known that in urodeles, such as newts, normal tissue dedifferentiates first before the new specific structure is reconstructed in the regeneration process [74]. Perhaps this was one of the reasons why Jonas [75] argued, though not totally unopposed [76], that the process of regeneration

could be seen as a kind of cancerous growth. Whether true tumor cells can be converted to normal cells if placed in the "regenerating field" is, of course, an interesting and important problem.

Rose and Wallingfold transplanted the tissue fragment of frog renal tumor into the limb of the newt *Triturus viridescens* [77]. After the transplant took hold and infiltrated the newt limb, the limb of the host was amputated leaving the tumor. In subsequent histological observations, frog cells were distinguished from newt cells by the size of the nucleus and the difference in stainability with haematoxylin. The results showed that regeneration occurred in the normal way in all cases, and that transplanted cells originating from frog tumor differentiated into muscle, cartilage and fibrous connective tissue and freely mingled with and spread in the host tissues. The cells of the renal tumor were epithelial in origin, but they apparently differentiated into many other directions. The results, however, remain controversial, since a reinvestigation, carried out by Ruben [78] gave negative results, i.e., the transplants kept their own characteristic tubuli *renalis* (=or uriniferous tubule) structures during regeneration and had not mixed with other tissues.

There was an interesting report that urodele epithelial tumor induced by treatment with MC differentiated into normal tissue spontaneously [79] but it is not clear to what extent the tumor disappeared or became differentiated. Recently, Tsonis [80] using newt papilloma, examined the effects of the presence of a tumor on the process of limb regeneration and the behaviour of the tumor cells in the regenerated tail. He observed that although the differentiation proceeded normally, the formation of the regenerative cone became retarded in tumor-bearing newts. When the tail with a tumor was amputated through the tumor, the tumor cells covered the surface of the wound but did not mix with normal epithelium and did not invade the blastema.

The problem of redifferentiation of tumor cells into normal tissue in the regenerating field needs to be reinvestigated. A clear distinction of transplanted tumor cells from host cells in transplantation studies is paramount for the correct interpretation of the results. Furthermore we need to give attention to the question whether tumor cells can be incorporated at all in the regeneration process.

The effects of chemical carcinogens on regeneration have been examined. Urodela obviously have a high resistance against carcinogens, and malformations rather than tumors occurred as a response. Tsonis and Eguchi treated forelimb blastema with a crystal of various carcinogens (about 5 μg), e.g. MNNG, 4-NQO, MC or BP for 7 days after amputation [81–83]. These carcinogens were not able to arrest the regeneration completely, but abnormal bones were formed or the regeneration was delayed. Abnormal limb regeneration can be classified into several types. For example, complete deficiency of both ulna and radius; abnormal regeneration and polymorphism of carpal bones, metacarpal bones and phalanges of fingers; hypertypic limb regeneration, and incomplete limb regeneration. Normally newts have four fingers in the forelimb and five in the hindlimb. When the left hindlimb was amputated and a small crystal of 4-NQO was put into 7-day blastema, the regenerated limb, developed an abnormal 6-fingered polymorphism, but a tumor was not formed.

The effects of carcinogens such as 4-NQO or MNNG on the cells of newt blastema are different from those on normal cells of other vertebrates. In newts the carcinogens show no carcinogenic activity, but only cause changes in cell movement or cell behaviour in the blastema. The blastema cells of newt are very resistant and stable against carcinogens. The relationship between the action mechanism of carcinogens in the regeneration process of urodeles and the reaction of blastema cells in the regeneration field provides the tumor scientist with a unique experimental system.

It is known that the iris in an eye also has strong regeneration power. When the lens is removed from an eye of a newt, cells of the upper part of the iris dedifferentiate, proliferate, form a lens vesicle, and bring about the regeneration of the lens. At this time, a strong carcinogen was administered into an eye ball after the removal of the lens. But, once again, no tumor was formed

as in the case of limb regeneration. Though only one lens is regenerated in normal regeneration, several lenses were regenerated following the administration of a carcinogen. The lens, regenerated while being treated with a carcinogen, is normal with regard to lens differentiation and transparency, but it seems almost impossible for the eye to function properly, considering there are several lenses in an eye [84]. This experimental system is very interesting if one desires to study the mechanisms of cell differentiation and cell reaction affected by carcinogens in lens regeneration.

2. Nuclear transplantation and potency of cell differentiation

Studies have been performed by means of the nuclear transplantation technique to determine whether the nucleus of a tumor cell possessed latent pluripotency and whether it had genes which enabled it to differentiate into various types of normal cells, tissues and organs. King and DiBerardino transplanted a nucleus from a Lucké renal tumor cell into an anucleated egg [85]. The nucleus was obtained from either proliferated tumor cells of primary tumorous tissue which were implanted into the anterior chamber, or from cells which were cultured *in vitro* for a short period. Out of the eggs with nuclear transplants, 1–5% reached the normal blastula state. In some of them development proceeded to late neurula and even larval state.

On the other hand, when the donor nuclei originated from normal kidney cells 3% of the eggs reached the blastula. The capacity to continue the process of cleavage was similarly developed in the nuclei from the tumor. However, when nuclei from blastula or gastrula were used as donors, 37% of all embryos reached the state of blastula and 40% of them developed into normal larvae. As for the arrest of the development, at some stage, of the embryos with nuclear transplants, it was shown that the chromosomes divided abnormally during the early stages of development and that the embryos, thereafter, failed to develop normally [86]. Although abnormal chromosomes occur frequently in embryos which developed from nuclei that originated from a differentiated cell, abnormal chromosomes were relatively rare in embryos which originated from nuclear transplants of undifferentiated cells. From these observations it was concluded that abnormal chromosomes in embryos coming from nuclear transplants reflected the degree of differentiation of donor nuclei prior to transplantation.

Then, to unambiguously show pluripotency of renal tumor nuclei, the nuclei were marked and made identifiable by being triploid [87, 88]. Since the nuclei of the host cells were diploid, the distinction between host nuclei and transplanted tumor nuclei was possible. First, triploid early embryos were obtained by the technique of low temperature treatment. These triploid embryos were infected with Lucké herpes virus. Embryos which proceeded development and yielded tumor cells in the pronephros were obtained. Next, the triploid tumor cell nuclei were injected into anucleated eggs. Then the eggs that had received triploid tumor nuclei proceeded development, but no tumor at all was formed. Moreover, to clarify these results, the nuclei from cultured triploid renal carcinoma cells were also transplanted [88].

Eggs with transplanted nuclei developed in such a way that 47% of them became blastulae, 17% and 20% became gastrulae and neurulae, respectively, and 3% developed into swimming larvae. The nuclei of these embryos were all triploid, and histologically, the cells differentiated into all organs of the body such as brain, spinal cord, optic cup and lens, somites, pronephros, midgut and so on. These results suggest that the nucleus of a tumor cell is genetically multipotent and can differentiate reversibly. Thus, using the nucleus of Lucké renal tumor cell, normal cloned larvae could be obtained. This is not only an important result, which agrees with observations on plant tumors [89] but also demonstrates a convenient experimental approach to study the expression of pluripotency of tumor cells and their ability to redifferentiate.

3. Effects of temperature

It has already been described that both in Lucké renal tumor and in newt papilloma, the abundance of the tumor depended notably on the

season [41–44, 52–56]. One of the causes of the seasonal change in tumor appearance is thought to be temperature. Especially, since amphibians are poikilothermic animals, they represent a convenient material to study the changes of tumor cells by means of alteration of temperature. In homoiothermic animals such as mammals, although it is possible to change the temperature regionally or even that of the whole body for a short period, it is difficult to change the body temperature from higher temperature to lower for any length of time. Amphibians allow such experiments to be performed and it is then possible to investigate the effects of temperature on the tumor, the regulatory mechanisms of the body and the properties of the virus.

Newts with papillomata of moderate size (2.5–3.5 mm in diameter) were chosen. They were divided into five experimental groups of different temperature conditions (4, 10, 13, 25 and 30°C) and bred under these controlled temperatures, while the diameters of the tumors were measured weekly [55, 56, 90]. As a control, papilloma-bearing newts were bred outdoors in the shade. The size of the tumor tended to increase gradually at 10°C and 13°C which are temperatures similar to those present in autumn, but it decreased notably both at 4°C (lower temperature) and at 25 and 30°C (higher). Newts have the ability to attenuate the tumor in their bodies; depending on changes in environmental temperature, they may even possess the ability to cure themselves and have the tumor regress.

Interestingly, the tumor regression occurred at both lower and higher temperatures, but it was found by histological examination that the way the regression occurred was different at both temperatures. At the higher temperature end the regression occurred more vigorously at 30°C than at 25°C. The size of the papilloma began to decrease soon after the animal was placed in the higher temperature. Cells of the upper layer of the tumor keratinized more actively than that of normal epidermis, i.e., shedding off tumor cells and size-reduction of tumor mass took place simultaneously. On the other hand, under conditions of the lower temperature movement of cells into the dermal layer was observed though tumor cells necrosed in part. The movement of cells was found at the earlier period soon after the change in temperature (within 4 weeks). However, the apparent size of the tumor did not become reduced in this early period. The reduction in size began after two months, and the tendency of the tumor to regress became more intense than at the higher temperature condition.

Generally, epithelial cells proliferate in the papilloma, but important changes in the dermal and pigment layers are lacking. However, at lower temperature, the number of cells in the epithelium decreased and simultaneously, that of the pigment layer increased. A down-growth of the epithelioma cells was observed at 4°C (low temperature treatment) [91]. The effects of temperature became evident much earlier. The mitotic index was strongly affected within as early as one week after the onset of temperature treatment. In the newts kept at 4°C or 30°C, the mitotic indices remained low (0.03–0.27) throughout the experimental periods, whereas under mild temperature conditions (10°C), the mitotic index of papilloma cells became significantly higher than at other temperatures. It is evident that the mitotic indices are closely related to the size of the newt papilloma [91].

When newts with regressing papillomata, caused by exposing them to lower or higher temperatures, were once again maintained at middle temperatures (10°C or 13°C), the tumor cells began to proliferate and increase the size of the tumor again. The growth of this tumor can be controlled or regressed reversibly by the effects of temperature. In addition to this effect, it has occasionally been observed that newts are likely to possess another method for curing a tumorous growth.

Generally, the tumor regresses gradually by depending on the change of temperature, but in some newts the mass of tumor disappeared almost abruptly from their bodies. This phenomenon may represent a form of "spontaneous therapy of tumor" in newts. It may be called "tumor cut-off" and could be comparable to the casting off of the tail in a lizard [74], and may be the most efficient method, and certainly fastest, to deal with a tumor. Practically, the process is achieved by

blood vessels being clogged up by blood cells and the subsequent prevention of the blood flow through the tumorous tissue. As a result, tumor cells necrose entirely and the mass of the tumor is removed from the root.

When it became obvious that newt papilloma is influenced by changes in temperature, it was thought that amphibians could serve as experimental material to study tumor regression, especially aspects of change and movement in tumor cells, and the contribution of virus in tumorous growth. However, to date almost no study has been performed with such objectives. For biochemical analysis, diamine and polyamine levels in spontaneous skin papilloma of newt were determined. In the papilloma putrescine was most abundant among the polyamines being nearly 5 times higher than that in the control skin [92].

Tumor bearing leopard frogs and the virus are not found in nature during summer. However, the latent existence of a virus can be confirmed by cold temperature treatment. Lucké renal tumor is, therefore, also thought to be a convenient system to analyse the effects of temperature on tumor cells and the role that the virus plays. Back to newts, it was pointed out earlier that they had papillomata predominantly in autumn, whereas Lucké renal tumor was present in large numbers twice a year, namely in spring and in autumn. If papillomas in newts would be controlled by means of temperature as the only causation factor, newt tumor ought to appear both in spring and in autumn. However, as the frequency of the tumor is actually low in spring, it suggests that factors promoting the tumor are not only temperature alone but others like, for example, hormones, growth factors or properties of the virus, too. As yet the different roles of these factors are not fully understood.

There is proof of the close relationship between temperature and cell motility and the cell movement of tumor cells. McKinnell *et al.* observed the distribution of microtubules in the cytoplasm of Lucké renal tumor cells, the established cell line PNKT-4B, the primary culture of renal tumor and, as a control, normal kidney cells of tadpole [93]. The cultures were kept at 20°C or 28°C and the microtubules were observed by the immunofluorescence method. In all of the three cultures, the microtubules were distributed regularly from the centre of the cell to the periphery, but when the cultures were kept at 7°C the distribution of microtubules in the tumor cells became irregular while normal cells remained unaffected. The microtubules of tumor cells quite unlike those of certain dermal cells in fish [94] become disordered by low temperature treatment similar to the disorder of microtubules seen in normal cells after application of a microtubule inhibitor. In Lucké renal tumor, metastasis formation occurs commonly at 28°C (77%), but much more rarely at low temperature (6%). It has also been suggested that the collagenase secreted by Lucké tumor *in vitro* explants degradated type 1 collagen at 30°C and was having an effect similar to that of temperature during metastasis formation [95]. However low levels of collagenase were also released at room temperature.

In those studies meant to illuminate the effects of temperature in newt papilloma the observed cell movements did not always agree with the results obtained on Lucké renal tumor. Nonetheless, amphibian tumor cells are excellent material to study the mechanisms of tumor formation, the complicated movement of cells at tumor growth and the phenomenon of regression. We are, therefore, convinced that human cancer research can only gain from the work, presently undertaken in various labs around the world, on amphibian tumor and regeneration.

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REFERENCES

1 Krontovsky, A. (1916) Comparative and Experimental Pathology of Tumors, Kiev, Bacteriol. Inst., (In Russian).
2 Sheremetieva, E. A. (1938) Rep. Inst. Zool., Acad. Sci. Ukr. S. S. R., **12**: 37–61 (In Russian).
3 Lucké, B. and Schlumbarger, H. G. (1949) Physiol. Rev., **29**: 91–126.
4 Duryee, W. R., Long, M. E., Taylor, H. C., McKel-

way, W. P. and Ehrmann, R. L. (1960) Science, **131**: 276–280.
5 Brunst, V. V. and Roque, A. L. (1967) J. Natl. Cancer Inst., **38**: 193–204.
6 Hadji-Azimi, I. and Fischberg, M. (1967) Rev. Suisse Zool., **74**: 641–645.
7 Hadji-Azimi, I. and Fischberg, M. (1971) Cancer Res., **31**: 1594–1599.
8 Mizell, M. (1969) Biology of Amphibian Tumor, Springer, Berlin, Heidelberg & New York, pp. 1–484.
9 McKinnell, R. G. and Ellis, V. L. (1972) Cancer Res., **32**: 1154–1159.
10 Wirl, G. (1972) Arch. Geschwulstforsch., **40**: 111–115.
11 Balls, M. and Clothier, R. H. (1974) Oncology, **29**: 501–519.
12 Khudoley, V. V. and Eliseiv, V. V. (1979) J. Natl. Cancer Inst., **63**: 101–104.
13 Khudoley, V. V., Anikin, I. V., Sirenko, O. A. and Pliss, G. B. (1979) Vopr. Onkol. (Leningrad), **25**: 70–75 (In Russian).
14 Eliseiv, V. V. and Khudoley, V. V. (1980) Vopr. Onkol. (Leningrad) **26**: 70–71 (In Russian).
15 Jaenisch, W. and Schmidt, T. (1980) Arch. Geschwulstforsch., **50**: 253–265.
16 McKinnell, R. G., DiBerardino, M. A., Blumenfeld, M. and Bergad, R. D. (1980) Results and Problems in Cell Differentiation, **11**: Differentiation and Neoplasia, Springer-Verlag, Berlin, Heidelberg & New York, 310 pp.
17 Schlumberger, H. G. and Lucké, B. (1948) Cancer Res., **8**: 657–753.
18 Waddington, C. H. (1935) Nature, **135**: 606–608.
19 Needham, J. (1936) Proc. R. Soc. B, **29**: 1577–1626.
20 Khudoley, V. V. (1982) Bull. Exp. Biol. Med., **92**: 1084–1085.
21 Lucké, B. (1934) Am. J. Cancer, **20**: 352–379.
22 Effron, M., Griner, L. and Benirschke, K. (1977) J. Natl. Cancer Inst., **59**: 185–198.
23 Balls, M. (1962) Cancer Res., **22**: 1142–1154.
24 Reichenbach-Klinke, H. and Elkan, E. (1965) II. Diseases of Amphibian, Acad. Press, New York.
25 Dawe, C. J., Harshbarger, J. C., Kondo, S., Sugimura, T. and Takayama, S. (1981) Phyletic Approaches to Cancer. Proc. 11th Int. Symp. Princess Takamatsu Cancer Res. Fund., Japan Sci. Soc. Press, Tokyo.
26 Okada, T. S. (1979) Cancer Cells, UP Biology Ser. 36, Tokyo Univ. Press, Tokyo, pp. 1–128 (In Japanese).
27 Kimura, I. (1984) Current Encyclopedia of Pathology, **9c**: 304–318, Nakayama-shoten, Tokyo (In Japanese).
28 Asashima, M. and Oinuma, T. (1985) Oncologia, **13**: 99–114 (In Japanese).
29 Brunst, V. V. (1968) Exp. Cell Res., **53**: 401–409.
30 Khudoley, V. V. and Mizgireuv, I. V. (1980) Neoplasma, **27**: 289–293.
31 Mizgireuv, I. V., Flax, N. L., Borkin, L. J. and Khudoley, V. V. (1984) Neoplasma, **31**: 175–181.
32 Oinuma, T., Seki, M. and Asashima, M. (1984) Proc. Jpn Acad., **60B**: 265–268.
33 Counts, C. L. III., Wilson, C. T. and Taylor, R. W. (1975) Herpetologica, **31**: 422–424.
34 Khudoley, V. V. and Eliseiv, V. V. (1979) J. Natl. Cancer Inst., **63**: 101–103.
35 Counts, C. L. III. (1980) Herpetologica, **36**: 46–50.
36 Woitkewitsch, A. A. (1959) Natürliche Mehrfachbildungen an Froschextremitäten. VEB Gustav Fischer Verlag.
37 Meyer-Rochow, V. B. and Koebke, J. (1986) Zool. Anzeiger, **217**: 1–13.
38 Rostand, J. and Darré, P. (1969) C. R. Soc. Biol., **163**: 2033–2034.
39 Lucké, B. and Schlumbarger, H. (1936) J. Exp. Med., **70**: 257–268.
40 Rafferty, K. A. Jr (1964) Cancer Res., **24**: 169–185.
41 McKinnell, R. G. and McKinnell, B. K. (1968) Cancer Res., **28**: 440–444.
42 McKinnell, R. G. (1969) In "Biology of Amphibian Tumors". Ed. by M. Mizell, Springer-Verlag, Berlin, pp. 254–260.
43 McKinnell, R. G., Gorham, E., Martin, F. B. and Schaad, J. W. IV. (1979) J. Natl. Cancer Inst., **63**: 821–824.
44 McKinnell, R. G., Gorham, E. and Martin, F. B. (1980) Am. Midl. Nat., **104**: 402–404.
45 Asashima, M., Sasaki, T. and Takuma, T. (1986) Proc. Jpn. Acad., Ser. B, **62**: 307–310.
46 Honma, Y. and Murakawa, S. (1967) Annot. Zool. Japon., **40**: 211–214.
47 Bryant, S. V. (1973) Cancer Res., **33**: 623–625.
48 Burns, R. E. and White, H. J. (1971) Cancer Res., **31**: 826–829.
49 Darquenne, J. and Matz, G. (1971) Bull. Soc. Zool. Fr., **96**: 352–353.
50 Rose, F. L. and Harschbarger, J. C. (1977) Science, **196**: 315–317.
51 Pfeiffer, C. J., Nagai, T., Fujimura, M. and Tobe, T. (1979) Cancer Res., **39**: 1904–1910.
52 Asashima, M, and Komazaki, S. (1980) Proc. Jpn. Acad., Ser. B., **56**: 638–642.
53 Asashima, M., Komazaki, S., Satou, C. and Oinuma, T. (1982) Cancer Res., **42**: 3741–3746.
54 Asashima, M. (1983) Animal and Nature, **13**: 15–19.
55 Asashima, M., Oinuma, T., Matsuyama, H. and Nagano, M. (1985) Cancer Res., **45**: 1198–1205.

56 Asashima, M. and Koyama, H. (1986) Jpn. J. Hyperthermic. Oncol., **2**: 359–370.

57 Placais, D. (1974) Bull. Soc. Zool. Fr., **99**: 283–295.

58 Lucké, B. (1938) J. Exp. Med., **68**: 457–468.

59 Mizell, M., Stackpole, C. W. and Halpern, S. (1968) Proc. Soc. Exp. Biol. Med., **127**: 808–814.

60 Zambernard, J. and Vatter, A. E. (1966) Cancer Res., **26**: 2148–2153.

61 McKinnell, R. G., Ellis, V. L., Dapkins, D. C. and Steven, L. N. Jr (1972) Cancer Res., **32**: 1729–1733.

62 Tweedell, K. S. (1967) Cancer Res., **27**: 2042–2052.

63 Mizell, M. (1969) In "Biology of Amphibian Tumors". Ed. by M. Mizell, Springer-Verlag, Berlin, pp. 1–25.

64 Tweedell, K. S. and Williams, D. C. (1976) J. Cell Sci., **22**: 385–395.

65 Naegele, R. F., Granoff, A. and Darlington, R. W. (1974) Proc. Natl. Acad. Sci., **71**: 830–834.

66 Nace, G. W. and Ostrovsky, D. S. (1977) J. Natl. Cancer Inst., **58**: 453–454.

67 Rubin, M. L. and Nace, G. W. (1966) Am. Zool., **6**: 510.

68 Hirayasu, T., Iwamura, Y. and Asashima, M. (1987) (to be submitted).

69 Asashima, M. and Oinuma, T. (1982) J. Fac. Sci. Univ. Tokyo, Sec IV., **15**: 151–158.

70 Shimada, K., Koyama, H. and Asashima, M. (1987) Zool. Sci., **4**: 287–294.

71 Balls, M. and Ruben, L. N. (1968) Prog. Exp. Tumors Res., **10**: 238–260.

72 Balls, M. and Ruben, L. N. (1964) Experientia, **21**: 241–296.

73 Spemann, H. (1942) Wilhelm Roux' Arch. Entwicklungsmech. Org., **141**(4): 693–769.

74 Alibardi, L. and Sala, M. (1983) Atti Mem. Acad. Patavita Sci. Lett. ed Atti, **95** (Pt II: Scienze Matematiche e Naturali): 101–151.

75 Jonas, A. D. (1985) Orientierungshilfen zur Psychotherapie in der Allgemeinpraxis-archaische Relikte in Psychosomatischen Symptomen (pp. 92–97). Edition Materia, Medica, Verlag Socio-medico, Grafelfing.

76 Meyer-Rochow, V. B. (1985) Selecta, **27**, 2309.

77 Rose, S. M. and Wallingford, H. M. (1948) Science, **107**: 457.

78 Ruben, L. N. (1956) J. Morphol., **98**: 389–404.

79 Seilern-Aspang, F. and Kratochwil, K. (1962) J. Embryol. Exp. Morphol., **10**: 337–353.

80 Tsonis, P. A. (1984) Can. J. Zool., **62**: 2681–2685.

81 Tsonis, P. A. and Eguchi, G. (1981) Differentiation, **20**: 52–60.

82 Tsonis, P. A. and Eguchi, G. (1982) Dev. Growth Differ., **24**: 183–190.

83 Tsonis, P. A. and Eguchi, G. (1983) Dev. Growth Differ., **25**: 201–210.

84 Eguchi, G. and Watanabe, K. (1973) J. Embryol. Exp. Morphol., **30**: 63–71.

85 King, T. J. and DiBerardino, M. A. (1965) Ann. N. Y. Acad. Sci., **126**: 115–126.

86 DiBerardino, M. A. and King, T. J. (1965) Dev. Biol., **11**: 217–242.

87 McKinnell, R. G., Deggins, B. A. and Labat, D. D. (1969) Science, **165**: 394–396.

88 DiBerardino, M. A., Mizell, M., Hoffner, N. J. and Friesendorf, D. G. (1983) Differentiation, **23**: 213–217.

89 Braun, A. C. (1965) Sci. Am., **213**: 75–83.

90 Asashima, M. (1984) Oncologia, **11**: 147–150.

91 Asashima, M., Seki, M., Kanno, H. and Koyama, H. (1986) Proc. Jpn. Acad., Ser. B., **62**: 83–86.

92 Matsuzaki, S., Kurabuchi, S. and Inoue, S. (1985) Zool. Sci., **2**: 131–134.

93 McKinnell, R. G., DeBruyne, G. K., Mareel, M., Tarin, D. and Tweedell, K. S. (1984) Differentiation, **26**: 231–234.

94 Obika, M. and Meyer-Rochow, V. B. (1986) Cell Tissue Res., **244**: 339–343.

95 Ogilvie, D. J., McKinnell, R. G. and Tarin, D. (1984) Cancer Res., **44**: 3438–3441.

96 Lunger, P. D., Darlington, R. W. and Granoff, A. (1965) N. Y. Acad. Sci., **126**: 289–314.